CLXXXV. THE MALE HORMONE. IV¹.

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UNTIL quite recently we have prepared the male hormone from urine acidified to Congo red and extracted with chloroform. The chloroform residue was steam-distilled and finally taken up with alkali. It was then observed that while steam distillation is an excellent procedure for getting rid of objectionable impurities, it also results in an appreciable loss in hormone activity. This was especially marked when superheated steam was used. Maximum activity was obtained only when the final residue could be completely dissolved. On many occasions this was not possible except by first treating the residue with *N* alkali and then with *N*/20 alkali. When these two separate solutions were subsequently combined, then obviously some of the active material separated out and was filtered off.

In repeating the above method of extracting the hormone, but using decidedly more acid, we discovered that the final product was even less soluble in alkali than before. However, the residue taken up in oil proved more potent than a comparable residue prepared by the use of less acid.

Since the use of more concentrated acid gave a more active product, but one less soluble in alkali, it was hoped that solubility could be enhanced by first boiling the chloroform residue with alkali, and then acidifying, steam distilling, etc. The results were interesting, but did not solve the problem of solubility in alkali.

At this stage we came across the papers of Butenandt [1929] who holds that the female hormone occurs in two forms, one having an open ring formation whilst the other is a lactone. To obtain the hormone completely alkali-soluble it is necessary to heat with alkali, so as to convert any lactone modification into the open ring form. On the other hand, heating with acid converts most of the substance into the lactone. Butenandt also states that if the material heated with alkali is extracted with ether, various impurities are removed, but the active material stays behind.

Having formed the opinion that the male and female hormones behave similarly towards chemical reagents, we now hoped that we could purify the

¹ Earlier papers have appeared in the *Proc. Soc. Exp. Biol. Med.* (1929), 26, 325 and 569, in the report of the *Thirteenth International Physiological Congress* (1929), p. 89, and in the *Amer. J. Physiol.* (1930), 92, 440.

male hormone by applying Butenandt's procedure with the female hormone. However, it was soon discovered that when the material obtained from male urine was heated with alkali and extracted with ether, most of the activity passed into the ether layer. Here for the first time one could observe a very sharp difference in chemical behaviour between the male and female hormones.

These results also suggested that possibly alkaline extraction of urine had advantages over acid extraction, particularly so in view of the work of Dingemanse [1930]. Actual experiments did not prove this to be the case. On the contrary, considerable loss of activity resulted when the urine was not acidified prior to extraction. It is probable then that the male hormone is present in the urine in two forms: one in the free state, and the other so combined or in such form as to be insoluble in chloroform, and only rendered soluble in this solvent by treatment (hydrolysis?) with acid.

It is probable that alkaline extraction of urine may be used to separate the male and female hormones¹, as Dingemanse points out; but our experiments show that the separation is only complete in so far as the female hormone is concerned, although by the alkali-ether treatment, outlined above, a better yield of the male hormone is obtained.

Out of a mass of experiments performed in connection with this work, we select one of the most recent, since it illustrates a number of points raised in this note.

In this experiment we started with 24 litres of mixed urine, obtained from men under 40 years of age. 6 litres of this urine were directly extracted with chloroform after the urine had become distinctly alkaline, the chloroform was evaporated, and the residue taken up with peanut oil (*a*). The remaining 18 litres were first acidified to Congo red, then strongly acidified (40 cc. conc. HCl per litre of urine), and extracted with chloroform under reflux. One-third of the chloroform extract (corresponding to 6 litres of urine) was evaporated, and the residue taken up with oil (*b*); two-thirds of the chloroform extract (corresponding to 12 litres of urine) was evaporated, heated with 10 g. NaOH in 50 cc. water for two hours, extracted repeatedly with ether, the ether extract divided into two equal portions, the ether in each case evaporated, and the one residue taken up with oil (*c*) and the other with diethylin (diethyl ether of glycerol) (*d*). In each case capons were injected with the equivalent of 300 cc. of urine per day. For each experiment four capons were used. The results were as follows:

(a) Percentage increase in comb growth in 5 days	...	12.6
(b) " " "	...	26.4
(c) " " "	...	19.5
(d) " " "	...	19.3

¹ The occurrence of male hormone in female urine and female hormone in male urine can probably be best explained by the occurrence of these hormones in foods. This view is supported by the fact that urine of even very old men still contains small, but appreciable quantities of the male hormone.

Roughly speaking, then, twice as much of the male hormone can be obtained from a strongly acidified urine as from alkaline urine.

Our results also lead to the view that ether extracts much of the male hormone from strongly alkaline solutions (obtained by extracting strongly acidified urine). According to Butenandt this is not true of the female hormone.

REFERENCES.

- Butenandt (1929). *Naturw.* 17, 879; *Deutsch. med. Woch.* No. 52.
Dingemanse (1930). *Abstr. Second Inter. Congr. Sex Research*, p. 46.